The effect of phenological variation in sex expression on female reproductive success in *Saxifraga granulata*[1]

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PREMISE OF THE STUDY: Differences in timing of flowering within and among protandrous plants shift the floral sex ratio from male to female dominance during the flowering season. Hence, the number of seeds produced by a single flower depends on traits of the flower itself (e.g., allocation to male and female function, position within an inflorescence, and flower size), as well as plant traits (e.g., timing of flowering, number of flowers, and plant height). Although it is clear that characteristics of individual flowers and whole plants can affect the number of seeds produced per flower, their relative importance for plant fitness remains largely unknown.

METHODS: We examined how phenological sex expression affected seed number per flower in two populations of the protandrous grassland herb *Saxifraga granulata*. Seed number was assessed for >1200 flowers and related to their position within an inflorescence, male and female phase duration, timing of flowering, number of flowers per plant, and plant height.

KEY RESULTS: Seed number within and among plants decreased significantly over time. Early lateral flowers were larger and produced more seeds in comparison to late lateral flowers, indicating that flower position significantly affected seed number through its combined effect on sex allocation, timing of flowering, and attractiveness.

CONCLUSIONS: Our results showed that female reproductive success of a single flower was best explained by its position within an inflorescence and that plant traits such as first flowering date and number of flowers per plant had a smaller impact on seed number per flower.

KEY WORDS first flowering date; flowering phenology; gender; protandry; reproductive ecology; seed production; sex allocation; within-plant variation

40 Plant species generally produce multiple flowers that often differ in shape and size (Herrera,
41 2009). As a result of these differences, the number of seeds per flower may vary substantially
42 not only among plants, but also among flowers of the same plant, implying that
43 characteristics of the flower itself as well as plant-level characteristics may affect seed
44 production per flower (Herrera, 2009; Ishii and Harder, 2012).

45 Most hermaphroditic plant species have mechanisms to separate female and male
46 organs in time (i.e., dichogamy) and space (i.e., herkogamy), to avoid inbreeding and pollen–
47 pistil interference (Charlesworth and Charlesworth, 1978; Bertin and Newman, 1993).
48 Separation of sexes in time (dichogamy) is a widespread floral strategy that occurs in many
49 outcrossing species (Barrett, 2003). Dichogamous species either start dispersing pollen before
50 stigmas become receptive (protandry) or start with the female phase before pollen dispersal
51 takes place (protogyny). In protandrous species, differences in timing of flowering typically
52 lead to a shift in floral sex ratio from male to female dominance during a population's
53 flowering season (Ishii and Harder, 2012). This shift in sex ratio may, in turn, induce
54 negative frequency-dependent selection, favoring early plants and early flowers within
55 inflorescences that invest in female function, and late flowers and late plants that invest in
56 male function (Brunet and Charlesworth, 1995; Ishii and Harder, 2012).

57 In protandrous plant species, the role of flowers as female and male organs is
58 therefore expected to vary not only among individuals (Devlin and Stephenson, 1987;
59 Klinkhamer et al., 1997; Wright and Barrett, 1999), but also within inflorescences (Brunet
60 and Charlesworth, 1995; Itagaki and Sakai, 2006; Herrera, 2009), and their importance is
61 likely to change during the course of the flowering season. Early flowers within
62 inflorescences generally have higher fruit and seed number than late flowers (Diggle, 1995),
63 and early-flowering plants generally produce more fruits and seeds than late-flowering plants

64 in temperate regions (Munguía-Rosas et al., 2011). This effect is stronger in plants with only
65 one reproductive event (i.e., annuals and monocarpic perennials) than in polycarpic
66 perennials, which may be due to stronger selective pressures on timing of flowering during
67 periods of favorable conditions for species with few reproductive events (Munguía-Rosas et
68 al., 2011).

69 Timing of flowering and shifts in floral sex ratio are not the sole factors that
70 determine female reproductive success. In plants that produce multiple flowers, seed number
71 can also be affected by the size and shape of flowers as well as by the attractiveness of a plant
72 (Herrera, 2009). Therefore, traits associated with pollinator attraction such as petal size,
73 number of flowers per plant, and plant height can also be expected to affect fruit and seed set
74 (Conner and Rush, 1996; Medel et al., 2003; Cariveau et al., 2004; Grindeland et al., 2005;
75 Sandring and Ågren, 2009; Parachnowitsch and Kessler, 2010; Sletvold et al., 2010; Karron
76 and Mitchell, 2012). Previous studies have shown that variation in fruit width was generally
77 higher within than among plants, indicating that floral characteristics substantially affect seed
78 number per fruit (reviewed in Herrera, 2009). However, few studies have assessed the
79 relative importance of phenological variation in sex expression and "attractiveness" on the
80 number of seeds per flower over the course of the entire flowering season.

81 We investigated variation in floral and plant characteristics in two populations of the
82 protandrous grassland herb *Saxifraga granulata* across the flowering season. We assessed
83 relationships between female reproductive success, floral sex expression, the position of a
84 flower within an inflorescence, flowering phenology, and plant traits associated with
85 pollinator attraction and determined their relative importance. We hypothesized that negative
86 frequency-dependent selection on sex expression is a major driver of variation in seed
87 number per flower in *S. granulata*. Hence, we predicted that:

(1) early flowers and early-flowering plants have an extended female phase and produce more seeds than late flowers and late-flowering plants;

(2) floral traits directly related to sex expression, such as position within an inflorescence and allocation to male and female phase duration, have a major impact on seed number per flower; and

(3) plant traits not directly related to sex expression, such as number of flowers per plant and plant height, are less important drivers of variation in seed number per flower.

MATERIALS AND METHODS

Study species and site—*Saxifraga granulata* L. ("meadow saxifrage") is a rosette herb that mainly occurs on mesic to dry grasslands in Western Europe and North Africa (Andersson, 1996). Data for this study were collected during the flowering season of 2013 in two populations of *S. granulata* in central Belgium: "Doode Bemde" (DB; 50°49′2.58″N; 4°38′47.38″E) and "Zoet Water" (ZW; 50°49′31.24″N; 4°38′55.01″E). Both populations were situated in mesic grasslands, were ~1 km apart, and were separated by a small forest patch. Both populations contained ~1000 flowering individuals and had the same level of genetic diversity, based on nine polymorphic microsatellite loci (H_S: 0.61 and 0.66, respectively) (van der Meer and Jacquemyn, 2015).

Saxifraga granulata can reproduce sexually as well as clonally by production of small bulbils at the base of the plant and has been described as being gynodioecious (Marsden-Jones and Turrill, 1947; Stevens and Richards, 1985; Stevens, 1988). In both study populations we found only one individual that was completely male-sterile and a few individuals showed signs of decreased male fertility such as one or more functionally sterile

anthers and/or relatively small anthers. The individuals with reduced male fertility produced significantly fewer flowers than other (hermaphroditic) individuals (GLMM with population as random effect: $z = -2.7$, residual df = 256, $P = 0.007$). Hence, it was not clear whether this male fertility reduction was related to a mutation (as expected in a gynodioecious species; Charlesworth and Charlesworth, 1978) or low plant vigor.

Individual ramets usually start flowering in May and flower until the beginning of June, producing flowering stems ≤57 cm in length. Flowering stems contain, on average, seven (SD = 3) small white flowers that are predominantly pollinated by unspecialized flies and solitary bees (Hansen and Molau, 1994). Flowers open sequentially, starting with the central flower of the inflorescence, followed by three early lateral flowers, after which three late lateral flowers open (Fig. 1). *Saxifraga granulata* is self-compatible, and individuals can self-fertilize geitonogamously because the female phase of early flowers generally overlaps with the male phase of late flowers of the same individual. Walisch et al. (2012) have shown that autogamous selfing in *S. granulata* is limited but can occur when flowers are bagged.

***Variation in sex expression*—**To study variation in floral and individual sex expression, we selected 260 plants before the start of the flowering season, 124 plants in population DB, and 136 plants in population ZW. To avoid studying clones, we selected individuals that were ≥40 cm apart and did not belong to the same spatial cluster. All plants were visited daily for 45 days (i.e., from the first day of flowering until fruit maturation) to record the number of open flowers and to determine floral sex expression of these flowers. Overall, the study included 1698 flowers on 260 plants. We followed phenological sex expression and assessed seed number of 1274 flowers. Another 424 flowers were collected to measure floral traits. A flower was considered to be in its male phase if at least one anther was dehisced and contained bright yellow pollen. When an anther has released all of its pollen, it usually

detaches from its filament. The end of the male phase generally coincided with the two stigmatic lobes detaching, exposing the stigmatic surface (i.e., the start of the female phase). A single flower flowered for 11 days on average (SD = 2).

On each plant, we marked one early lateral and one late lateral flower (Fig. 1) that were used to estimate pollen production and were eventually collected to measure several floral characteristics. When plants did not produce late lateral flowers, we only selected an early lateral flower. In total, 258 early and 166 late lateral flowers were selected for floral measurements. We assessed pollen production by collecting 2 of 10 anthers from all selected early and late lateral flowers before they opened. The anthers were first dried, then incubated in 25 μL HCl at 50°C for ≥2 hr, and finally 75 μL lactophenol (10%) was added. To enhance staining of the pollen grains, the anthers were incubated once more at 50°C for 5 min. The number of pollen grains in 10 μL of the solution was estimated using a Neubauer-improved counting chamber (Marienfeld Superior, Lauda-Königshofen, Germany). All pollen grains in the four largest squares of 1 × 1 mm were counted. Every sample was loaded onto the counting chamber twice, and the average amount of pollen grains per 1 × 1 mm was used as a measure for floral pollen production. *Saxifraga granulata* produces ~600 ovules per capsule (Hansen and Molau, 1994). Unfortunately, because of the tiny size of seeds and ovules, we were unable to accurately count the number of ovules per capsule in the present study.

***Plant and floral characteristics*—**Early and late lateral flowers that were selected for floral measurements were collected when they were in their female phase with fully developed stigmas. The length and width of one petal and one sepal, length of four filaments, and length of two styles were measured for every collected flower. We also determined plant height (from ground to tallest flower) of all studied individuals toward the end of the flowering season, when plants were fully grown. Mature fruits were collected just before they would

159 split open and release their seeds. All fruits were dried and seeds were hand counted. Seed
160 weight was estimated by dividing collective mass of all seeds in a fruit by the number of
161 seeds per fruit.

162 ***Timing of flowering*—**To study the effect of timing of flowering on sex phase duration and
163 the number of seeds per flower, we divided the plants into early-, peak-, and late-flowering
164 individuals. In population DB, plants started flowering between 1 and 17 May; in population
165 ZW, plants started flowering between 2 and 19 May. We divided the flowering season, for
166 both populations separately, into three equal groups based on the number of days. We then
167 assigned plants to one of the groups (early-, peak-, or late-flowering) according to their first
168 flowering date. We also calculated the degree of flowering synchrony within both
169 populations to quantify overlap in flowering times between individuals (Augspurger, 1983).
170 Augspurger's flowering synchrony index varies from 0 (no overlap) to 1 (complete overlap).

171 ***Data analysis*—**To study variation in sex expression and temporal changes in female
172 reproductive success within and between individuals we used linear mixed models (LMMs)
173 and generalized linear mixed models (GLMM) from the r-package "lme4" (Bates et al.,
174 2014). We used LMMs when the response variable was approximately normally distributed,
175 such as sex phase duration, petal size, and seed weight. When the response variable was not
176 normally distributed—for instance, when studying seed number or the number of flowers per
177 plant—we used GLMMs with family set to "poisson" and link function "log." Furthermore,
178 to account for similarities between flowers of the same plant and plants within a population,
179 we included "plant" and "population" as grouping variables with a random intercept in all our
180 analyses concerning flowers, while we only included "population" as a grouping variable
181 with a random intercept in our analyses based on individual plants.

182 First, we used LMMs to investigate whether flower position within an inflorescence

had an impact on floral traits, including male and female phase duration, filament size, style length, pollen production, petal size, and sepal size. To analyze whether flowers had a significantly longer male than female phase duration, we performed an LMM and included "flower" as a grouping variable with a random intercept as well as "plant" and "population" that were used in most analyses. To study whether sex phase duration differed between early-, peak-, and late-flowering individuals, we also used LMMs. Second, GLMMs were used to examine the effect of flower position and timing of flowering on seed number. To assess whether plants produced more seeds when they flowered more synchronously, Augspurger's synchrony index (calculated for both populations separately) was related to seed number using a GLMM. The same analysis was used to investigate whether seed number per plant was significantly related to first flowering date. However, since first flowering date can be highly correlated with other plant traits such as number of flowers per plant, plant height, and petal size (Ehrlén, 2015), we corrected for indirect effects via covariance by including these variables in the model. We also studied whether the position of a flower within an inflorescence affected seed number and seed weight using a GLMM and an LMM, respectively.

Finally, we investigated which floral or plant trait (i.e., flower position within an inflorescence, first flowering date, number of flowers per plant, and plant height) most strongly influenced the number of seeds per flower. To do so, we first standardized all floral and plant traits within population, to be able to compare regression coefficients and to account for differences between populations. We then performed a standard GLM with the number of seeds per flower as response variable and the standardized plant traits as explanatory variables (i.e., multiple regression). We also calculated correlation coefficients between the standardized plant traits used in the multiple regression (i.e., start of flowering, number of flower per plant, and plant height).

208 All analyses were performed using R version 3.0.2 (R Development Core Team,
209 2013).

210 **RESULTS**

211 ***Variation in sex expression*—**Plants began flowering on 1 May, and flowering lasted until
212 June 8, with peak flowering between the 19th and 25th days (Fig. 2, dashed line). Because of
213 the protandrous life history of *S. granulata* and the long male phase of flowers, the floral sex
214 ratio was male-biased at the start of the flowering season until after peak flowering (Fig. 2,
215 solid lines). Only in the last 4 days of the flowering season was the floral sex ratio female-
216 dominated. Individuals showed large variation in male and female phase duration within
217 inflorescences (Table 1) and between individuals (Table 2). Flowers spend 2 to 12 days in
218 their male phase and 1 to 9 days in their female phase. Male phase duration was, on average,
219 4.2 days longer than female phase duration (t = 68.2, residual df = 2540, $P < 0.001$). Late
220 lateral flowers produced significantly less pollen per flower in comparison to early lateral
221 flowers (Table 1). Variance partitioning of the results of the LMM studying the effect of
222 flower position on pollen production showed that differences in pollen production between
223 plants explained most of the observed variation (64%), whereas differences within
224 inflorescences explained 36% of the observed variation (t = −2.8, residual df = 419, P =
225 0.005; Table 1). Hence, pollen production was more variable between plants than within
226 inflorescences. We found no variation in pollen production between populations.

227 Central flowers generally invested more in female function, because the male phase
228 duration of these flowers was significantly shorter than the male phase of early and late
229 lateral flowers (Table 1). As a result, most central flowers quickly became female. We also
230 found significant differences in sex allocation within inflorescences between early-, peak-,
231 and late-flowering individuals. Central flowers of early-flowering plants had an exceptionally

short male phase (4.4 days; SD = 1.2), whereas late lateral flowers of late-flowering individuals had a short female phase (1.8 days; SD = 0.7) (Table 2). Late-flowering plants invested, on average, 20–39% of flowering time in female phase duration, whereas early-flowering plants invested, on average, 23–49% of flowering time in female phase duration (Fig. 3). Early lateral flowers also had significantly larger petals, sepals, and sex structures than late lateral flowers (Table 1).

***Timing of flowering and seed production*—**Flowering onset was negatively related to seed number per plant, which implies that plants that flowered in the beginning of the flowering season produced significantly more seeds than plants that started flowering late in the season ($z = -119.0$, residual df = 256, $P < 0.001$). This effect remained significant when we corrected for indirect effects via other plant traits (i.e., number of flowers, plant height, and average petal size; $z = -52.8$, residual df = 246, $P < 0.001$). The observed differences in sex allocation between central, early, and late flowers in early-, peak-, and late-flowering individuals were associated with significant differences in seed number (Table 2). A single central flower could contain as many as 1221 seeds but produced 527 seeds on average (SD = 217). Compared to lateral flowers, central flowers produced more, heavier seeds (Table 1). Moreover, central flowers of early-flowering plants produced significantly more and heavier seeds than central flowers of peak- and late-flowering individuals (Table 2). We found the same decline in seed number in early and late lateral flowers of early-, peak-, and late-flowering plants (Table 2), but seed weight of late lateral flowers did not decline significantly over time. Variance partitioning of the results of the GLMM studying the effect of flower position on the number of seeds per flower (Table 1) indicated that differences in number of seeds per flower within plants explained most of the variation (88%), while differences among plants accounted for 12% of the total variation. The average number of seeds per flower did not differ between populations.

Augspurger's index for flowering synchrony was 0.85, on average, in population DB and 0.83 in population ZW and was negatively related to seed number ($z = -110.7$, residual df = 256, $P < 0.001$; Fig. 4). Plants that flowered during peak flowering produced fewer seeds than plants that flowered less synchronously (e.g., plants that flowered early in the season).

***Floral vs. plant traits*—**The number of seeds produced by a single flower was most strongly affected by its position within an inflorescence, rather than by plant-level characteristics (Fig. 5). First flowering date, number of flowers per plant, and plant height affected the number of seeds per flower to a lesser extent (Fig. 5). Flower position and start of flowering negatively affected seed number, which indicates that early flowers and early-flowering plants produced more seeds than late flowers and late-flowering plants. Start of flowering was also negatively correlated with number of flowers per plant ($r = -0.31$, $t = -5.2$, residual df = 256, $P < 0.001$; Fig. 5), which indicates that early-flowering plants produced significantly more flowers than plants that flowered late in the season. Plant height was positively correlated with number of flowers per plant ($r = 0.55$, $t = 10.6$, residual df = 256, $P < 0.001$; Fig. 5), showing that tall plants generally contained more flowers than short plants.

DISCUSSION

We investigated how variation in female reproductive success of flowers was related to within- and among-plant characteristics across the entire flowering season of the protandrous grassland herb *Saxifraga granulata*. Our results showed that flower-level traits were more important than plant-level traits in explaining variation in the number of seeds per flower in this species.

***Variation in sex expression within inflorescences*—**Floral sex ratios shifted from male to female dominance during the flowering season. The male phase of this protandrous species dominated the largest part of the flowering season, which lasted ~40 days. Only during the

281 last 4 days, >50% of the flowers were female. Our results also showed that individual plants
282 displayed large variation in male and female function within inflorescences. Central flowers,
283 for example, had a relatively short male phase and quickly became female in comparison to
284 lateral flowers. Variation in male and female function within inflorescences of *S. granulata*
285 was consistent with expectations based on its protandrous nature, which may lead to negative
286 frequency-dependent selection on early flowers to invest in female function (Brunet and
287 Charlesworth, 1995). Similar variation in sex allocation within inflorescences has also been
288 shown in other protandrous species. In *Delphinium glaucum*, *Lobelia sessilifolia*, *Narthecium*
289 *asiaticum*, *Schiedea salicaria*, and *Stylidium armeria* early-opening flowers (low or terminal
290 flowers) invested more in female instead of male function whereas late flowers (top or lateral
291 flowers) showed the opposite trend (Ishii and Sakai, 2002; Hiraga and Sakai, 2007; Sakai et
292 al., 2008; Brookes and Jesson, 2010; Ishii and Harder, 2012). In some of these species, the
293 number of ovules declined over time, whereas pollen production remained constant or
294 declined at a slower rate. Hence, maleness increased over time because of an increase in the
295 pollen/ovule ratio rather than an increase in pollen production in late flowers (Ishii and Sakai,
296 2002; Hiraga and Sakai, 2007; Brookes and Jesson, 2010).

297 ***Flowering phenology*—**Timing of flowering significantly affected seed number per plant,
298 which suggests that it may be subject to selection. This is in line with the results of
299 Andersson (1996), who showed that seed set per flower was highest for early-flowering
300 individuals of *S. granulata* as with congener *S. stellaris* (Sandvik et al., 1999). However, it is
301 not clear whether timing of flowering is subject to direct pollinator-mediated selection or is
302 the result of indirect selection or environmental covariance (Ehrlén, 2015). Individuals
303 growing at favorable microsites may have access to more resources and, therefore, flower
304 earlier and produce more flowers and seeds. Many studies have shown that early-flowering
305 plants tend to be larger and produce more flowers, potentially explaining why most

306 correlative studies find selection for early flowering (Ehrlén and Münzbergová, 2009; Forrest
307 and Thomson, 2010). In the present study, we corrected for indirect effects via covariance of
308 the number of flowers per plant, plant height, and average petal size, but still found a
309 significant effect of first flowering date on seed number. Moreover, density-dependent
310 processes may also play an important role in selection on flowering time. Earlyand late-
311 flowering plants should have fewer potential mates than plants that flower midseason, but
312 they generally receive pollen from more distant donors, reducing biparental inbreeding
313 (Elzinga et al., 2007; Ison and Wagenius, 2014). This is in line with the relationship between
314 flowering synchrony and seed number found in the present study. Individuals that flowered
315 during peak flowering produced fewer seeds than individuals that flowered early or late in the
316 season. Furthermore, Walisch et al. (2012) have shown that geitonogamous crosses decreased
317 seed number in *S. granulata*— which, given that this species can propagate clonally, may
318 lead to strong negative effects of biparental inbreeding. Selection on early flowering also
319 involves both mutualistic and antagonistic interactions. Predispersal seed predation may
320 affect selection on first flowering date (Elzinga et al., 2007; Kolb et al., 2007). However, in
321 the present study, <1% of all fruits were damaged by herbivores, which indicates that
322 selection pressure on early flowering acting through predispersal seed predation was probably
323 limited. Temporal changes in pollinator abundance or community may have affected seed
324 number as well (Rafferty and Ives, 2011). Finally, timing of different life-cycle events can
325 lead to temporal trade-offs (Ehrlén, 2015). For instance, timing of flowering influences the
326 timing of subsequent events, such as dispersal, germination, and offspring performance. Even
327 though it is clear that flowers of early-flowering plants had an extended female phase
328 duration and produced significantly more seeds than late-flowering individuals, we cannot
329 completely rule out the possibility that selection on first flowering date may also occur via
330 selection on other life-cycle events.

***Floral and plant traits*—**The number of seeds per flower was most strongly related to the position of a flower within an inflorescence, while timing of flowering and plant traits associated with pollinator attraction (i.e., number of flowers per plant and plant height) also affected the number of seeds per flower, but to a lesser extent. This result may be a manifestation of frequency-dependent selection on sex allocation toward female function in early flowers. There are several ecological mechanisms that may have contributed to the observed decline in seed number from central to late lateral flowers. In protandrous species, the sexual neighborhood becomes female dominated at the end of the flowering season, increasing competition and pollen limitation (Bartkowska and Johnston, 2014), which may have been strengthened by decreased attractiveness of late lateral flowers due to decreased pollen production and smaller petal size (Elle and Carney, 2003). Seed number may also have been reduced as a result of ovule, flower, and fruit abortion (Stephenson, 1981). In general, plants preferentially allocate resources to earlier, more basal flowers (Wyatt, 1980; Stephenson, 1981), which indicates that floral architecture itself may affect female reproductive success of a flower. In this sense, late flowers merely serve a bet hedging function, providing a buffer for delayed fruit production due to variation in pollinator abundance (Forrest and Thomson, 2010).

Moreover, decreasing resource availability over time (Stephenson, 1981; Medrano et al., 2000) and in space (Stephenson, 1981; Diggle, 1995) may initiate abortion of ovules, flowers, and fruits, further contributing to the observed decline of seed number in late lateral flowers. Our observations showed that ~1% of the flowers, mainly late lateral flowers, wilted before onset of the female phase, whereas male phase duration was not shorter than average, indicating that these plants aborted the flowers after pollen dispersal but before investment in ovule fertilization. Late flowers of *S. granulata* may also produce fewer ovules and, as a result, produce fewer seeds. Hansen and Molau (1994), for example, found a linear

relationship between the number of ovules and the number of seeds in capsules of *S. granulata*. Although we were not able to assess ovule production, the observed decline in pollen production and seed number from early to late lateral flowers may indicate that ovule production decreased over time as well, which may have led to a situation where ovules, rather than pollen, were the limiting factor for seed number in late lateral flowers (Harder and Aizen, 2010). Finally, lateral flowers also had a higher chance of receiving pollen from the same plant through geitonogamous pollen transfer than central flowers, because there is only one central flower and usually several early and late lateral flowers per plant. To discriminate between effects of negative frequency-dependent selection, architectural effects, resource limitation, decreased ovule production, and selfing, additional experiments should be performed.

CONCLUSION

Our results show that individual plants displayed large variation in male and female phase duration within inflorescences and between individuals and that the floral sex ratio of the population shifted from male-biased at the start of the flowering season to female dominated at the end of the flowering season. Concomitantly, the number of seeds produced by flowers and plants decreased over time, and this decrease was related to floral as well as plant-level characteristics. In accordance with the results of Herrera (2009), we showed that the variation in seed number per flower was highest within, rather than among, individuals and was best explained by the position of a flower within an inflorescence. However, more research is needed to unravel the precise ecological mechanisms that determine within- versus among-plant variation in female reproductive success.

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502

TABLE 1. Floral characteristics (means ± SD) in relation to flower position within inflorescences of *Saxifraga granulata*. To account for variation between populations and plants, we included population and plant as grouping variables with random intercepts.

	Male phase (days)	Female phase (days)	Seed number	Seed weight (mg)	Pollen grains (per 1×1 mm)	Filament size (mm)	Style length (mm)	Petal size (mm)	Sepal size (mm)
Central flowers	5.8 ^{A} (± 1.5) *N* = 257	3.7 ^{B} (± 1.4) *N* = 257	527 ^{C} (± 217) *N* = 256	0.025 ^{B} (± 0.005) *N* = 254	NA	NA	NA	NA	NA
Early lateral flowers	7.8 ^{B} (± 1.4) *N* = 639	3.5 ^{B} (± 1.5) *N* = 639	256 ^{B} (± 182) *N* = 635	0.018 ^{A} (± 0.027) *N* = 552	12.9 ^{B} (± 3.6) *N* = 258	6.0 ^{B} (± 0.8) *N* = 258	5.5 ^{B} (± 0.9) *N* = 257	89.0 ^{B} (± 18.0) *N* = 253	8.7 ^{B} (± 1.6) *N* = 258
Late lateral flowers	7.7 ^{B} (± 1.4) *N* = 377	2.2 ^{A} (± 1.1) *N* = 377	40 ^{A} (± 80) *N* = 375	0.013 ^{A} (± 0.007) *N* = 109	12.1 ^{A} (± 3.2) *N* = 166	5.5 ^{A} (± 0.7) *N* = 164	4.6 ^{A} (± 1.2) *N* = 163	72.1 ^{A} (± 18.6) *N* = 163	6.4 ^{A} (± 1.4) *N* = 165
test statistic	$F = 367$	$F = 201.9$	$\chi^2 = 124071$	$F = 16.5$	$F = 8.0$	$F = 145.5$	$F = 108.1$	$F = 170.3$	$F = 416.6$
model df	2	2	2	2	1	1	1	1	1
residual df	1267	1267	1261	909	419	417	415	411	418
P	< 0.001	< 0.001	< 0.001	< 0.001	0.005	< 0.001	< 0.001	< 0.001	< 0.001

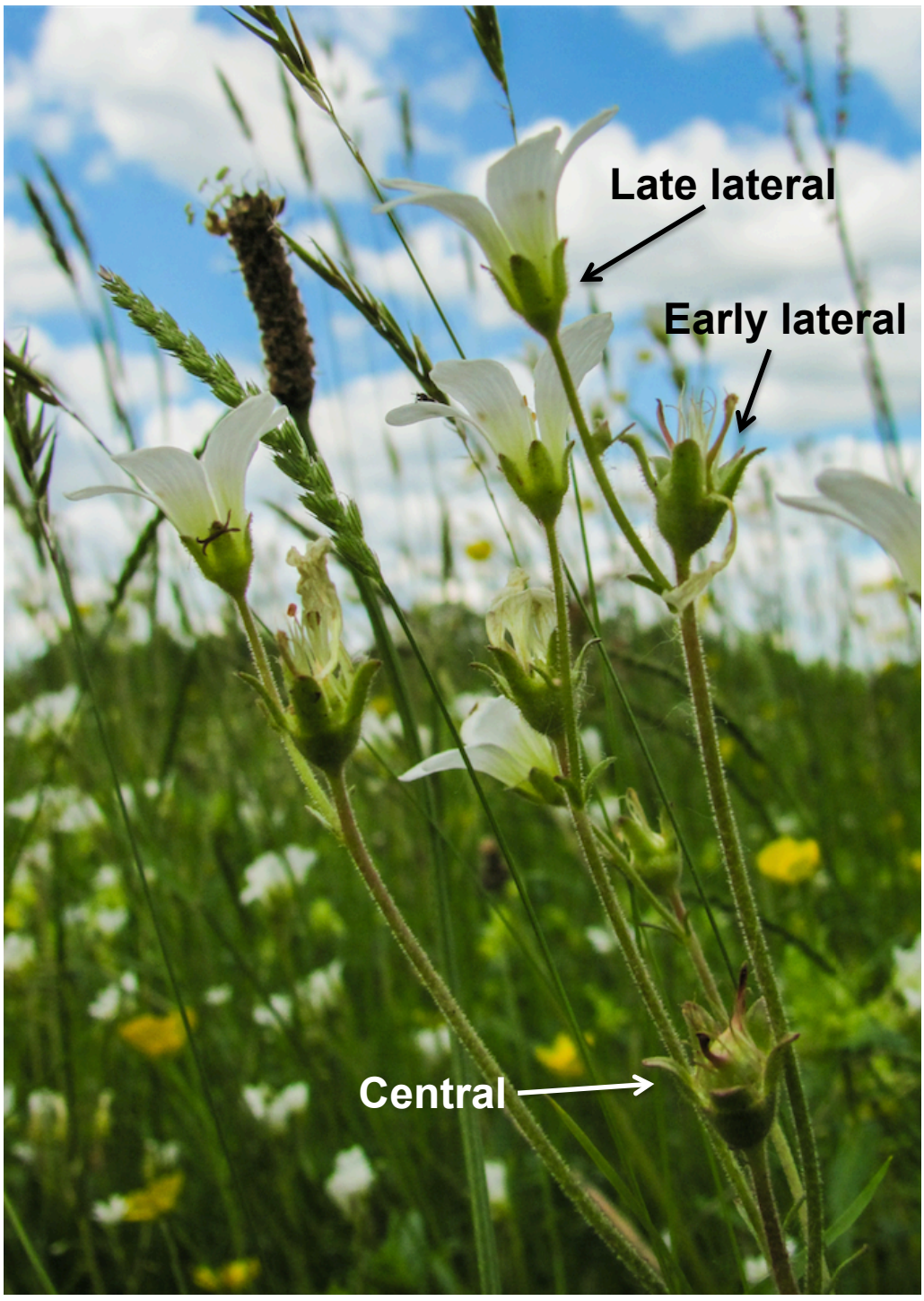
Note: *N* = number of samples, NA = not available, and df = degrees of freedom. Letters accompanying means and SDs for each flower position indicate the outcome of Tukey's multiple comparison test for mixed models; means that share the same letter within a column do not differ significantly ($\alpha = 0.05$).

511 **TABLE 2.** Differences in male and female phase duration, seed number, and seed weight
512 (means ± SD) within inflorescences of early-, peak-, and late-flowering indi- viduals of
513 *Saxifraga granulata*. To account for variation between populations and plants, we included
514 population and plant as grouping variables with random intercepts.

	Timing of flowering	Central flowers	Early lateral flowers	Late lateral flowers
Male phase (days)	Early	4.4 (± 1.2) ^{A} *N* = 83	7.2 (± 1.2) ^{A} *N* = 213	8.1 (± 1.3) ^{B} *N* = 183
	Peak	6.5 (± 1.2) ^{B} *N* = 144	8.2 (± 1.4) ^{B} *N* = 347	7.5 (± 1.5) ^{A} *N* = 171
	Late	6.6 (± 1.2) ^{B} *N* = 30	7.9 (± 1.0) ^{B} *N* = 79	6.9 (± 1.2) ^{A} *N* = 23
	F	88.0	30.2	16.0
	model df	2	2	2
	residual df	251	633	371
	P	< 0.001	< 0.001	< 0.001
Female phase (days)	Early	4.3 (± 1.4) ^{B} *N* = 83	4.3 (± 1.6) ^{B} *N* = 213	2.3 (± 1.0) ^{B} *N* = 183
	Peak	3.3 (± 1.3) ^{A} *N* = 144	3.2 (± 1.4) ^{A} *N* = 347	2.1 (± 1.1) ^{AB} *N* = 171
	Late	4.1 (± 1.6) ^{B} *N* = 30	3.1 (± 1.1) ^{A} *N* = 79	1.8 (± 0.7) ^{A} *N* = 23
	F	9.8	36.0	4.2
	model df	2	2	2
	residual df	251	633	371
	P	< 0.001	< 0.001	0.02
Seed number	Early	560 (± 241) ^{C} *N* = 82	314 (± 190) ^{C} *N* = 212	57 (± 96) ^{C} *N* = 182
	Peak	533 (± 199) ^{B} *N* = 144	236 (± 169) ^{B} *N* = 344	27 (± 65) ^{B} *N* = 170
	Late	407 (± 200) ^{A} *N* = 30	190 (± 172) ^{A} *N* = 79	2 (± 7) ^{A} *N* = 23
	χ^2	1252.1	994.3	186.7
	model df	2	2	2
	residual df	251	630	370
	P	< 0.001	< 0.001	< 0.001
Seed weight (mg)	Early	0.027 (± 0.004) ^{C} *N* = 81	0.022 (± 0.046) ^{C} *N* = 193	0.013 (± 0.006) *N* = 75
	Peak	0.024 (± 0.004) ^{B} *N* = 143	0.015 (± 0.004) ^{B} *N* = 293	0.013 (± 0.007) *N* = 33
	Late	0.022 (± 0.006) ^{A} *N* = 30	0.012 (± 0.005) ^{A} *N* = 66	0.007 (± 0.003) *N* = 2
	F	13.0	30.3	0.7
	model df	2	2	2
	residual df	248	546	104
	P	< 0.001	< 0.001	0.5

515 *Note: n* = number of samples and df = degrees of freedom. Letters accompanying means and SDs for each
516 flower position indicate the outcome of Tukey's multiple comparison test for mixed models between early-,
517 peak-, and late-flowering plants per flower position; means that share the same letter within a column do not
518 differ significantly ($\alpha = 0.05$).

519



520

521 **FIGURE 1** Floral architecture of an inflorescence of *Saxifraga granulata*. The central flower

522 is fruiting, the early lateral flowers have just wilted, and the late lateral flowers are open.

523

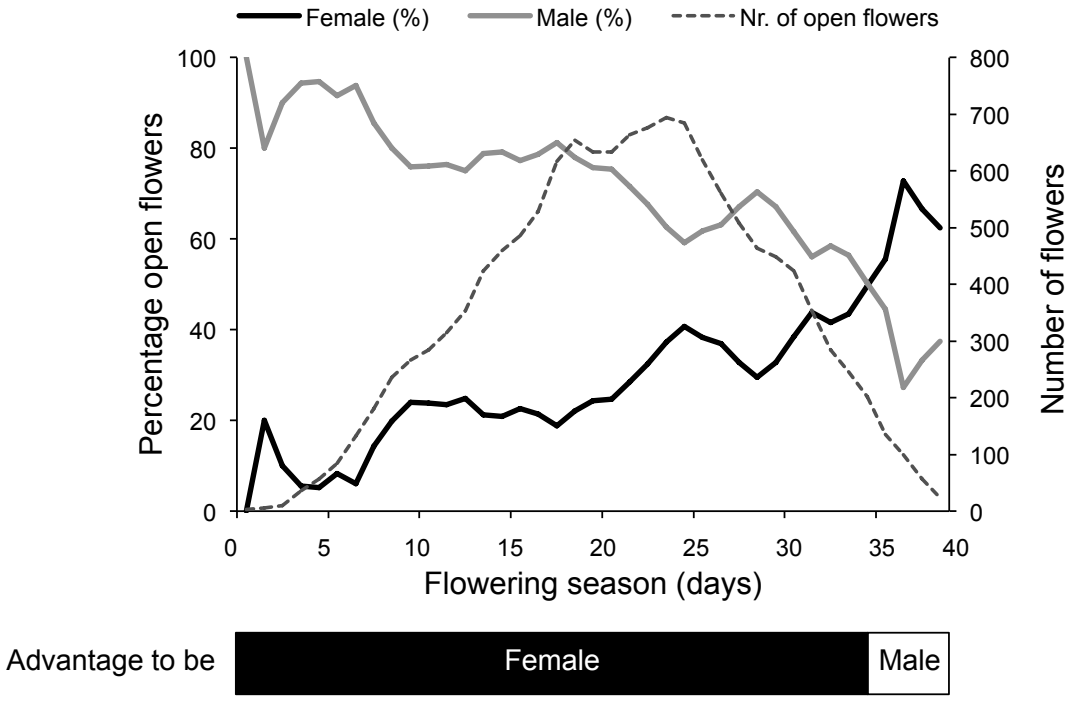
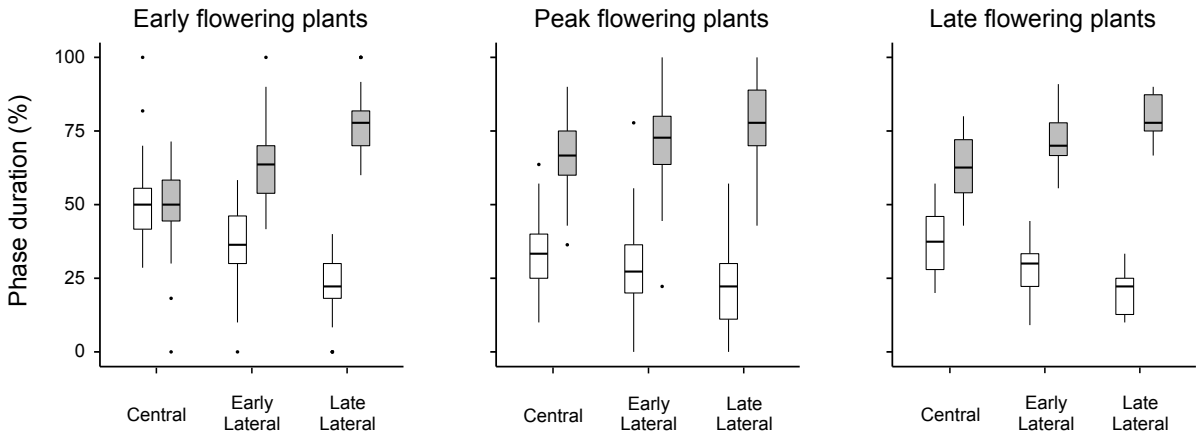


FIGURE 2 Shift in floral sex ratio during the flowering season of *Saxifraga granulata*, including percentages of open flowers that are in their male phase (gray solid line) or their female phase (black solid line) and the total number of open flowers (dashed line).

530



531

532 **FIGURE 3** Male (gray columns) and female (white columns) phase duration of flowers at
533 different positions for early-, peak-, and late-flowering individuals of *Saxifraga granulata*.

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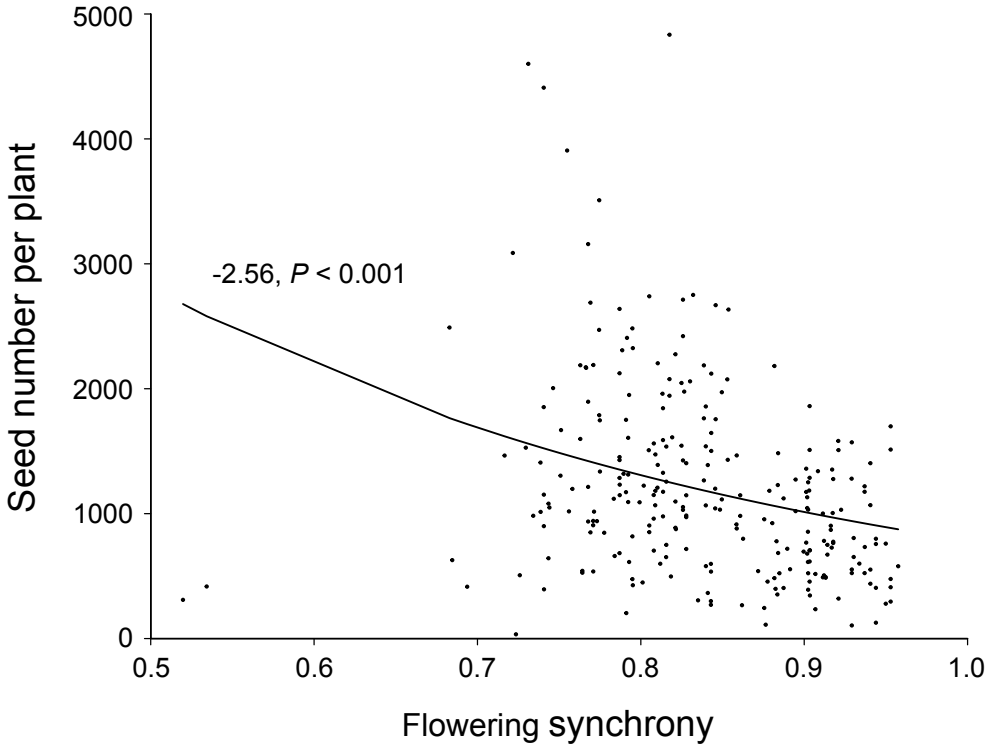

FIGURE 4 Relationship between flowering synchrony and seed number. Augspurger's flowering synchrony index varies from 0 (no overlap) to 1 (perfect flowering synchrony).

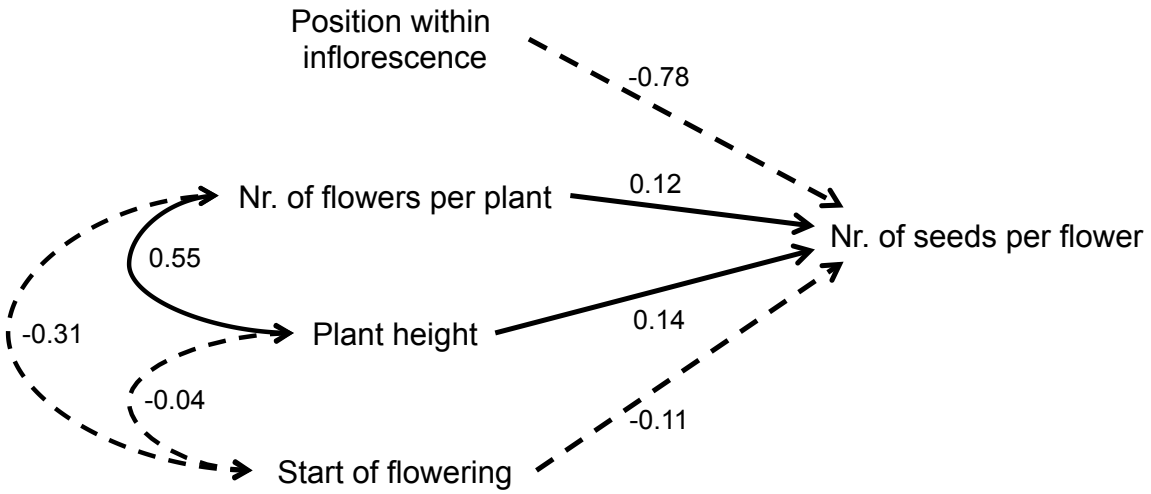

FIGURE 5 Results of the multiple regression analysis of flower position, number of flowers per plant, plant height, and start of flowering on the number of seeds per flower (straight lines) and correlations between plant traits (curved lines). Solid lines indicate a positive and dashed lines a negative coefficient.